

Gut

Leading article

Growth factors and the liver

The capacity of the liver to regenerate after severe viral or drug induced hepatitis, or after massive partial hepatectomy, is remarkable. Experimentally, the most dramatic and widely studied example of this phenomenon is regeneration after the performance of a 70% partial hepatectomy in rats, in which normal liver mass is restored within 10 days. Similar, though slower, regeneration occurs in humans. The possibility that hepatocyte regeneration could be enhanced therapeutically has been an enticing prospect for some years, and recent advances in our understanding of the processes controlling hepatocyte proliferation bring the prospect excitingly close to realisation.

After experimental partial hepatectomy the liver regenerates by the division of existing adult cells rather than by stem cell proliferation. There is a surge of DNA synthesis in hepatocytes, which peaks at 24 hours after the resection. After a further 24 hours there is a surge of DNA replication in the non-parenchymal cell population. The latter process is less well understood, but fascinating interactions between the main non-parenchymal cell types, Kupffer cells and sinusoidal endothelial cells, and hepatocytes are now emerging.

Although regeneration may be influenced by factors such as increased metabolic demand after loss of functioning liver mass, the critical events in initiating hepatocyte regeneration seem to entail growth factors interacting with specific receptors on cell surfaces.

Historically, two main theories dominated growth factor control of hepatic regeneration. The first was that reduction in liver mass stimulated the production of positive growth factors to initiate replication and repair. The second was that the normal liver was actively prevented from proliferation by a chalone, a locally produced inhibitor of growth; partial hepatectomy lowered the concentration of the inhibitor, and growth then occurred until the normal inhibitory tone was restored. The story that emerges now synthesises these two theories, outlining a complex interaction of stimulatory and inhibitory influences on hepatocyte growth.

A few years ago it seemed likely that epidermal growth factor (EGF) would emerge as the major stimulator of hepatic regeneration. EGF, a peptide of molecular weight approximately 6000, is a growth factor for several epithelial tissues, first recognised by its ability to mature ophthalmic tissues in newborn mice.¹ Receptors specifically binding EGF are present at high density on hepatocyte plasma membranes and within the cell.² When in vitro EGF combines with its receptor, DNA synthesis is stimulated within 18 hours. Signal transduction for DNA synthesis probably occurs by a process of autophosphorylation and internalisation of the

receptor.³ There is a high capacity uptake system for EGF in periportal areas of the lobule,⁴ where regeneration is pronounced. Exogenously labelled EGF administered at the time of hepatic regeneration is transported with its receptor to the hepatocyte nucleus.⁵

It was, however, difficult to show the final link in the chain linking EGF to hepatic regeneration after partial hepatectomy – an increase in the amount of EGF present in the circulation or the liver. Only minimal increases in the circulation were reported. This paradox was resolved by the demonstration that transforming growth factor α (TGF α), which has 30 to 40% sequence homology with EGF, can also bind to the EGF receptor and initiate hepatocyte replication in vitro.⁶ Furthermore, TGF α is generated in the liver shortly after partial hepatectomy. Mead and Fausto showed an increase in TGF α mRNA in hepatocytes of rat liver after partial hepatectomy, reaching levels ninefold greater than normal, and postulated an autocrine loop by which enhanced TGF α production in regenerating hepatocytes stimulated hepatocyte proliferation.⁶ The amount of TGF α mRNA returned to normal by four days. But, despite the powerful effect of TGF α in stimulating DNA synthesis, and evidence of its enhanced production after partial hepatectomy, recognition of the role of this growth factor by no means completes the story of the stimulation of hepatic regeneration. The time course of TGF α mRNA production, peaking at the time of peak DNA synthesis, is too long for the initiation of that process. TGF α might also therefore act on regeneration at the time that hepatocytes have made the transition from G0 to G1 and entered the cell cycle.^{7,8}

Other recent studies have identified at least three other growth factors that can either initiate or enhance hepatocyte proliferation which are generated in the liver or found in higher quantities in the circulation after partial hepatectomy. The most important of these is hepatocyte growth factor (hepatotrophin, probably identical with hepatopoietin A), in particular because this substance seems to be specific for hepatocytes. Hepatocyte growth factor (HGF) was described in the serum of rats by Nakamura *et al*⁹ 24 hours after partial hepatectomy, and the isolation procedure indicated that the molecule has affinity for heparin, a property shared by several growth factors.¹⁰ Selden *et al* reported the presence of a similar substance in serum from patients 24 hours after partial hepatectomy.¹¹ The substance has now been purified both from plasma from patients with fulminant hepatic failure and from rat platelets. It is of high molecular weight and is a highly active stimulator of DNA synthesis in hepatocytes, being active at concentrations as low as 1 ng/ml.

The sequence of human HGF was published by two groups recently.^{12,13} The substance hepatopoietin A purified by different techniques by workers at Duke University is identical.¹⁴ Close similarities between rat, rabbit, and human HGF, and the fact that its action is not species specific, have permitted rapid progress in showing the changes in HGF expression during regeneration. Although still the subject of controversy, work from two groups indicates that HGF is generated in the liver,^{13,15} probably in non-parenchymal cells,¹⁶ and the peak of mRNA expression, 10 hours after partial hepatectomy, falling by 24 hours, makes this protein a strong candidate for an initiator of DNA synthesis in the hepatocyte after partial hepatectomy.¹⁷ It has been shown recently that messenger RNA for HGF is strongly expressed in fetal liver tissue compared with normal adult tissue, suggesting for the first time that similar mechanisms of liver growth occur both during liver development and in liver repair after damage.¹⁵ Once purified material is available in adequate amounts, rapid advances are likely in characterising the HGF receptor and second messenger events leading to DNA synthesis. So far it seems to have no interactions with the EGF receptor.¹⁸

Heparin binding growth factor, otherwise known as acidic fibroblast growth factor I, unlike HGF, does not show specificity for liver cells. None the less it too is generated in the liver within a few hours of partial hepatectomy.¹⁸ It can stimulate hepatocyte proliferation in vitro in the presence of heparin, and in addition has an interesting interaction with EGF; although it decreases the response of hepatocytes to EGF when added simultaneously in vitro,¹⁹ it expands a regenerating cell population which may subsequently be a target for the action of EGF or TGF α . One role suggested for this protein is priming hepatocytes for further stimulation by their EGF/TGF α receptor. Heparin binding growth factor I stimulated hepatocytes are less sensitive to the powerful inhibitor of hepatocyte proliferation TGF β . Heparin binding growth factor I may have a dual role of initiating replication and overcoming local inhibitory influences. It has been suggested that it may be the same substance as hepatic stimulatory substance, a partially purified substance of molecular weight 26 000, which was purified from regenerating liver and is capable of enhancing the rate of regeneration of liver which has undergone minor degrees of partial hepatectomy.²⁰

Additional factors which must eventually be fitted into the full picture of hepatocyte regeneration include hepatopoietin B, a very low molecular weight substance found in the serum 24 hours after partial hepatectomy,²¹ and the sympathetic nervous system, as sympathetic denervation diminishes hepatic regeneration and norepinephrine enhances the action of EGF.²² There are also as yet uncharacterised growth factors capable of stimulating hepatocyte growth released from Kupffer cells isolated from regenerating liver.²³ Insulin and glucagon were once accorded a major role in hepatocyte proliferation, in particular after whole animal experiments in which pancreatectomy and diversion of portal blood were shown to strikingly diminish liver regeneration. Currently, however, they are thought to play a cooperative part in the process of hepatocyte proliferation, enhancing the effects of other mitogens.

Liver regeneration, like all good things, must come to an end. A major role is emerging for the non-parenchymal cells as controllers of proliferation of the hepatocyte population. While some of the positive growth factors already mentioned may be generated in non-parenchymal cells, the major inhibitor of hepatocyte proliferation, TGF β 1, is strongly expressed in non-parenchymal cells from 24 hours after partial hepatectomy until the process of liver regeneration ceases. The TGF β peptides are a family of growth factors, initially purified from platelets, which can have either

stimulatory or inhibitory effects on proliferation, depending on the tissues investigated.

Carr *et al* showed that TGF β from platelets could inhibit EGF induced stimulation of hepatocyte DNA synthesis,²⁴ and by a process which was not one of competitive inhibition at the EGF receptor. Subsequently, Braun *et al* showed that TGF β is strongly expressed in non-parenchymal liver cells after partial hepatectomy, particularly in the sinusoidal endothelial cells.²⁵ In addition, a low level of TGF β expression is normally present, and it may be that this low production prevents the response of the resting liver to the levels of various positive growth factors that are normally present in the liver and the circulation. Indeed, a postulated role of heparin binding growth factor I is to overcome this resting tone of TGF β immediately after partial hepatectomy.

It is unlikely that TGF β is the only cell mechanism operating to limit the regenerative process or to prevent growth in normal liver. At least one other inhibitory growth factor, distinct from TGF β , can be isolated from liver cytosol,²⁶ and cytokines potentially released from reticulo-endothelial cells or macrophages can also inhibit hepatocyte proliferation.²⁷ The factors controlling the proliferative response of non-parenchymal cell populations, both as stimulators and to limit that process, remain strikingly under-explored. The reductive approaches of cell and molecular biology, defining the actions of individual growth factors on individual cells, have proved to be major advances in our understanding of the process of liver regeneration. A clear message from the study of the synthetic capacity of hepatocytes, however, is that cells behave differently when in contact with other cell types and supporting matrices compared with their behaviour in isolated cell cultures. Understanding the full picture of hepatic regeneration requires an integrative approach, exploring the relation between different cell types and different growth factors in vitro.²⁸ In future the interaction with molecules, such as scatter factor,²⁹ which act on cell to cell interaction and the organisation of tissues will need to be addressed. It is already clear that potential tools for enhancing liver regeneration in severe hepatitis or after major surgery have been defined.

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